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Pymetrozine, a Fast-Acting and Selective Inhibitor of Aphid Feeding. *In-situ* Studies with Electronic Monitoring of Feeding Behaviour

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Abstract: Pymetrozine, a pyridine azomethine compound, represents a novel insecticide with a selective activity against homopteran insects. It acts in a unique way: aphids are not knocked down on contact but seem to die of starvation. This implies an effect of pymetrozine on feeding behaviour. The aim of the present work was to elucidate how and at which step pymetrozine interferes with the complex mechanisms underlying phloem feeding. The effect of pymetrozine, applied in various ways, on different phases of stylet penetration and feeding activity of individual aphids was studied using the Electrical Penetration Graph technique (EPG). Initial choice experiments indicated that pymetrozine does not have a deterrent or antifeedant action. Topical application (150 ng pymetrozine mg^{-1} fresh weight) inhibited stylet insertion into the plant. When injected, less than 30 ng mg^{-1} was sufficient to produce the same effect. When pymetrozine was systemically applied *via* plant spraying or root uptake, aphids started feeding normally. After some time, however, they withdrew their stylets from the phloem and walked around with unaffected locomotion. At low doses aphids eventually recovered and resumed feeding. High doses, however, irreversibly disrupted feeding and prevented stylet reinsertion. Aphid motility was not affected up to an estimated haemolymph concentration of 1 mM pymetrozine. Aphids which eventually stopped feeding on pymetrozine-treated plants showed EPGs with distorted salivation/ingestion patterns. It is concluded that pymetrozine does not have a general toxic effect on aphids but selectively interferes with the nervous regulation of feeding behaviour which consequently results in death due to starvation after a few days.

Key words: pymetrozine, insecticides, aphids, feeding behaviour, EPG technique

1 INTRODUCTION

Although most of the 4400 aphid species in the world are not of economic importance, they are all phytophagous insects and about 100 species cause considerable damage in agriculture or horticulture either by feeding on the vascular system or by transmission of plant viruses.[1] Only a few aphicides exist that spare natural enemies such as parasitoids and predators which can reduce aphid numbers under field conditions.[2] These products belong to the traditional insecticide groups (e.g. carbamates and organophosphorus compounds) with a considerable risk of becoming ineffective due to the build-up of resistance.[3] Aphids have a high reproductive rate and an efficient dispersal strategy.[4] Hence, there is a need for a safe and selective aphicide that fits into integrated pest management programs and keeps the crop protected against immigrating aphids.

Pymetrozine (Fig. 1) is a selective insecticide, the basic structure of which is a pyridine azomethine. It is effective against aphids, whiteflies[5] and hoppers and can be used in integrated pest management programmes.[6]

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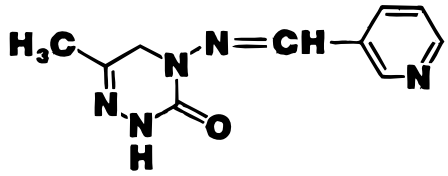

Fig. 1. Structural formula of pymetrozine.

There is no knock-down effect: the aphids remain alive and mobile for some time but do not seem to feed. The same result occurs after ingestion of sap from plants treated with pymetrozine, because of its systemic action.[7] As this behavioural effect does not suggest a deterrent action, some other possibilities for the mode of action of pymetrozine have to be considered.

The prevention of feeding could be due to (a) disruption of the sequence of behavioural steps in the search for an adequate feeding site on the host plant; (b) inhibition of stylet activities during penetration toward the phloem elements; (c) inhibition of cibarial muscles involved in food intake; (d) interference with essential metabolic processes, resulting in inability to feed.

To determine the mode of action, the Electrical Penetration Graph (EPG) technique was used to record distinct phases of penetration and feeding activity in individual aphids. These phases should be well-defined. In consort with the definitions made by Tjallingii[8–10] we use the following terminology: *Feeding behaviour* in aphids includes all activities related to sap feeding, probing and exploratory locomotion to find new feeding sites. *Probing behaviour* is part of feeding behaviour, including probing and non-probing intervals on the same plant. *Probes* or *stylet penetrations* are synonyms used for the periods of stylet contact with the plant. *Ingestion* will refer to all sap intake into the gut. In aphids, it includes *drinking* from xylem elements and *sap feeding* from phloem sieve elements. Definitions and terminology of waveform patterns, waveforms and waves reflecting these activities is according to Tjallingii.[11,12]

During penetration, aphids probe a number of cells before reaching the phloem and injecting saliva or ingesting sap through separate canals in the stylet bundle. With the EPG technique, mechanical stylet activity, punctures of plant cells, salivation, and active and passive ingestion can be recorded and distinguished.[9,11]

Inability to feed normally, as observed after application of pymetrozine should therefore be reflected in changes in the normal sequence of EPG patterns.

2 MATERIALS AND METHODS

2.1 Aphids

Colonies of *Aphis fabae* Scopoli were reared on broad bean plants (*Vicia faba* L.), *Macrosiphum euphorbiae* (Thomas) on potato plants (*Solanum tuberosum* L.), *Myzus persicae* (Sulzer) (biotype M_2 = sensitive, M_3 = resistant to organophosphorus insecticides) on Chinese cabbage (*Brassica cernua* (Thbg Forbes Hemst)) and lettuce (*Lactuca sativa* L.) plants and *Aphis gossypii* Glover on cucumber plants (*Cucumis sativus* L.). All plants were kept in a glasshouse at 19($\pm$ 2)°C. Both winged and wingless morphs were used in the experiments. Winged, parthenogenetically reproducing females (alatae) were produced by crowding 10 adult aphids in a clipcage of 20 mm diameter and removing daily batches of offspring to continue development on fresh plants or artificial diets. Unless stated otherwise, 20 wingless and 20 winged aphids were used for each test.

2.2 Treatments

2.2.1 Choice between plants treated and untreated with pymetrozine

Rooted lettuce and bean plants were placed in nutrient solutions containing 0, 40 and 100 mg litre^{-1} of pymetrozine for uptake *via* the roots (see Section 2.2.6). After 24 h the plants were placed in a Plexiglass cage of 50 (height) × 35 × 35 cm and 50 winged *A. fabae* (with beans) or *M. persicae* (with lettuce) were released in the upper part of the cage. After 2 and 24 h the numbers of aphids on each plant were counted. The experiment was replicated three times. Loglinear models were fitted to the observed counts with species, trial, treatment and the interaction between species and treatment as explanatory variables.

2.2.2 Topical application

Both winged and wingless (apterous) aphids were tested. Aphids were positioned on a vacuum-operated manipulation table under a stereomicroscope. A microneedle fixed to a micromanipulator was adjusted to touch the dorsum of the insect. Stock solutions of pymetrozine in dimethylsulfoxide (DMSO) were appropriately diluted with acetone. Pymetrozine was topically applied in a volume of 25 nl to an aphid of 350 μg with a pressure-operated system with optical calibration. All dosages were based on this body weight and adjusted for deviating weight to be presented as ng mg^{-1} fresh weight. Controls received the solvent (DMSO + acetone) only. Both the pymetrozine-treated and the solvent-treated aphids were immediately prepared for the EPG technique which took about 5 min. Mortality and reproduction data were obtained with 10 clipcages each containing 10 aphids that received a small droplet of silver paint on the thorax as described in Section 2.3. The mortality data were fitted to logistic regression models with variance proportional to binomial variance and species, treatment and their interaction as explanatory variables. Effects were assessed using the mean deviance ratio, resulting in approximate F-tests. Pair-

wise differences between treatment means on the logit scale were tested using a *t*-test. *T*-tests were also used for the remaining variable differences between treatments within species. Differences between treatments were considered to be significant at $P < 0{\cdot}05$. Analyses were performed using the Genstat (Genstat 5 Committee, 1993) statistical program.

2.2.3 Injections into the haemolymph

For micro-injections, self-made calibrated glass needles were used with a solid tip and a side-opening. This device prevents obstruction and facilitates recalibration after tip resharpening. Aphids were not anesthetized, but placed ventral side upwards on the same manipulation table as mentioned under Section *2.2.2*. Stock solutions were made up in DMSO and diluted with aphid saline (Na^+, 391; K^+, 19; Ca^{2+}, 11; Cl^-, 302 mM). Usually, the injected volume was 2 nl containing not more than 80 ml litre^{-1} DMSO; for an aphid of 350 μg this is about 1/200 of the body volume corresponding to about 1/70 of the body fluids that comprise 1/3 of the total volume. Controls were injected with the same amount of solvent. Aphids were individually weighed on a Cahn 4700 microbalance and dosages were adjusted to body weight.

2.2.4 Diet feeding

Two flow chambers, containing about 2 ml of chemically defined diet nr 144 or 148,[13] were covered with Parafilm. Diets could be replaced or chemicals added under sterile conditions within 5 s, while the aphids remained feeding. A ground electrode for EPG registration was inserted through the bottom of each chamber, in contact with the diet.

2.2.5 Foliar application

Plants were sprayed with pymetrozine solutions on a turntable until runoff at 80 and 100 mg litre^{-1}. Controls were sprayed with water only. Aphids (*A. fabae*, *A. gossypii*, *M. euphorbiae* and *M. persicae*) were allowed to settle on the plants after 24 h. Mortality and reproduction tests and statistical operations were as described in Section *2.2.2*. Plants were treated identically to those used for the EPG technique.

2.2.6 Root application

Plants were reared in aerated nutrient solutions (NO_3^-, 11·67; $H_2PO_4^-$, 0·79; HPO_4^{2-}, 0·40; SO_4^{2-}, 6·92; K^+, 7·18; Ca^{2+}, 8·98; Mg^{2+}, 4·04 mequiv.) to avoid damage at the start of the experiments. For systemic root uptake, plants of the same weight were transferred to small glass jars containing solutions of pymetrozine. Care was taken that the solution was taken up in 24 h (the volume depending on the plant size) and it was then replaced by fresh distilled and aerated water. Mortality and reproduction tests were started 24 or 48 h later and statistical analysis was as described in Section *2.2.2*. Plants were treated identically to those used for the EPG technique.

2.3 Electrical recording of penetration behaviour

Basically, the EPG technique used was as described by Tjallingii.[9] It is a direct current system with adjustable voltage source, supplying an electrical potential to the substrate. The equipment was housed in a constant-temperature room (20(±1)°C), insulated from electrical noise and equipped with high frequency fluorescent light and DC-operated spot illumination, providing at least 120 μE m^{-2} s^{-1}. After being starved for 2 h the aphids were connected to a gold wire of 20 μm diameter with a small droplet of water-soluble silver paint on the dorsal side of the thorax. The gold wire was connected to the probe of a DC amplifier (input resistance 1 GΩ). The ground electrode was placed in the pot soil, the root medium or connected to a chamber for artificial diets. The EPG signals were displayed on a two-channel memory oscilloscope and a high frequency (HF) chart recorder (Graphtec WTR 77/A). At the same time, the EPG signals were stored on the hard disk of a computer through an A/D conversion. Such EPG files can be processed statistically and signals calibrated with the HF recordings. The aphids were lowered onto or removed from a leaf by means of a micromanipulator under electrical control.

3 RESULTS

3.1 Choice experiments

With winged adults, there were no significant differences between the numbers of aphids alighting on untreated and treated plants after 2 or 24 h (Table 1). After 2 h some of the aphids were on the walls and roof of the cage. After 24 h all *M. persicae* and 48 out of 50 *A. fabae* had settled on a plant, but they did not discriminate between untreated plants and those treated with 40 or 100 mg litre^{-1} pymetrozine.

3.2 Topical application

Untreated aphids started probing within minutes of being placed on the plant. They lowered the tip of their proboscis onto the epidermis, inserted their stylets and produced a normal EPG (Fig. 2). Both *M. persicae* and *A. gossypii* treated topically with 50 ng of pymetrozine per individual did not probe within 5 min of application. Though the aphids started walking on the leaf surface, stylet insertion was effectively blocked. Within

TABLE 1
Number of Aphids Alighted on Treated and Untreated Plants (Released 50) on Three Replicates

Pymetozine conc. (*mg litre*$^{-1}$)	*Number alighted* (± *SE*)[a]			
	M. persicae		A. fabae	
	2 h	*24 h*	*2 h*	*24 h*
Untreated	11 (± 0·56)	15 (± 1·35)	13 (± 0·60)	16 (± 1·40)
40	9 (± 0·50)	18 (± 1·48)	11 (± 0·56)	14 (± 1·31)
100	13 (± 0·60)	17 (± 1·44)	10 (± 0·53)	18 (± 1·48)

[a] Differences between treatments were not significant (approximate *F*-test, $P > 0{\cdot}05$).

6 h, however, most aphids recovered and resumed probing (Table 2). The EPGs now produced showed the normal sequence of patterns and E2 waveforms (Fig. 2). However, the E2 waveforms, which reflect sap intake, were not sustained. The aphids either switched from E2 to C pattern or completely withdrew their stylets. After a few days the aphids died, some of them having produced a few larvae. *A. fabae* showed similar behaviour after receiving the same dose, although this species took more time to recover (7–12 h) and insert the stylets and survived as long as 48 h.

Application of 100 ng pymetrozine resulted in complete disruption of penetration behaviour. None of the treated aphids inserted their stylets. Some were observed tapping their proboscis on the leaves, but no electrical contact was made with the plant. Most aphids died within 24 h.

3.3 Micro-injections

When injected into the haemolymph of *M. persicae*, pymetrozine evoked the same effects as after topical application, though at lower doses. At 7 ng per adult, effects were comparable to topical application of 50 ng. As this way of application is directly into the body fluids, 7 ng per individual of 350 μg equals 21 ng μl^{-1} or 21 mg litre^{-1}, corresponding to $c.10^{-4}$ M pymetrozine in the haemolymph. The aphids still aggregated at the leaf veins protruding from the surface, but did not

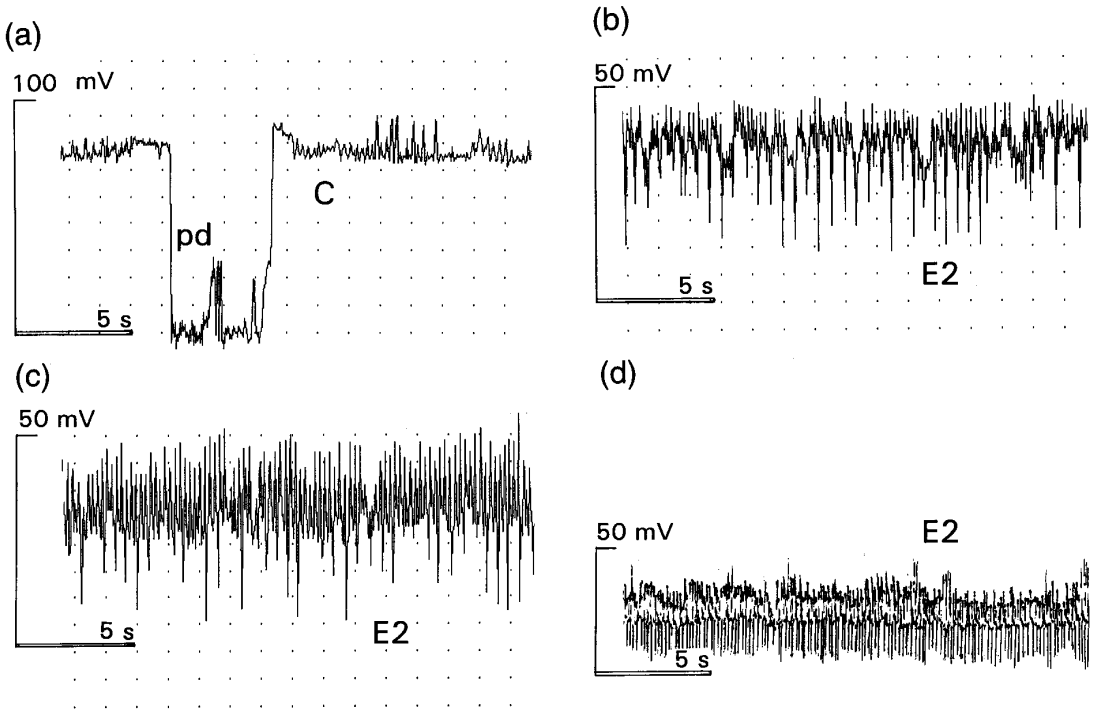

Fig. 2. (a) EPG computer registration of a control *M. persicae* feeding on Chinese cabbage, showing C pattern (pathway activities in the mesophyll) and a potential drop (pd, puncture of the plasmalemma), (b) passive phloem ingestion waveform E2, (c) E2 waveform of an aphid recovered from application of a low dose (25 ng) of pymetrozine, (d) as in (c), but recorded with a Graphtec high frequency chart recorder.

TABLE 2

Effect of Topical Application of Pymetrozine on Penetration Behaviour of Wingless Morphs of *Myzus persicae* and *Aphis gossypii* Recorded with the EPG Technique and on Mortality and Fecundity

	Mean value ($\pm SE$)[a]								
Aphid species (*host plant*)	M. persicae (M_2)[b] (*Chinese cabbage*)			M. persicae (M_3)[b] (*Chinese cabbage*)			A. gossypii (*cucumber*)		
Dosage (*ng mg*$^{-}$ *fresh weight*)	*0*	*150*	*300*	*0*	*150*	*300*	*0*	*150*	*300*
Time to first penetration (min) ($n = 20$)	1·5 ($\pm$1·2)	280 ($\pm$46·5)	~ [c]	1·4 ($\pm$0·8)	300 ($\pm$62·4)	~	1·8 ($\pm$1·5)	220 ($\pm$57·2)	~
Time to reach the phloem at first successful penetration (min)	16 ($\pm$10·3)	> 300	~	12 ($\pm$7·6)	> 300	~	18 ($\pm$9·1)	> 300	~
Interval to second penetration	3 ($\pm$1·8)	> 120	~	4 ($\pm$2·3)	> 150	~	3 ($\pm$1·6)	> 120	~
Longest E2 waveform	> 10 h	36 min	~	> 10 h	22 min	~	> 10 h	15 min	~
Mortality in 24 h (%; $n = 100$)	2 ($\pm$1·3)	12 ($\pm$3·6)	89 ($\pm$3·5)	3 ($\pm$2·1)	10 ($\pm$3·9)	94 ($\pm$2·2)	3 ($\pm$1·5)	14 ($\pm$3·3)	85 ($\pm$4·5)
Average daily offspring per aphid	2·0 ($\pm$0·19)	0·8 ($\pm$0·10)	0	2·2 ($\pm$0·21)	1·5 ($\pm$0·18)	0	2·3 ($\pm$0·18)	1·0	0 ($\pm$0·11)

[a] All data ($n = 10$) of treated aphids (second and third columns of each species) are significantly different from their controls and the third columns from the second (*t*-tests, $P < 0{\cdot}05$).

[b] M_2 and M_3 are different biotypes.

[c] Aphids did not probe during 6-h recording periods.

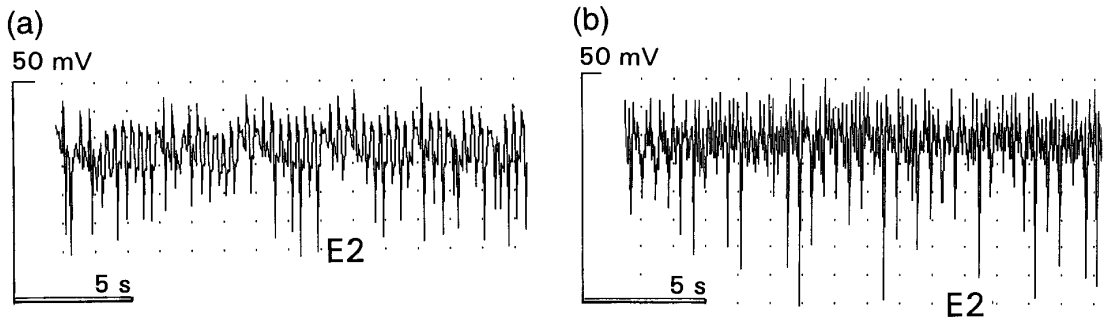

Fig. 3. (a) E2 waveform of a recovering *M. persicae* 8 h after injection of 7 ng pymetrozine (approximate final concentration of 10^{-4} M in the haemolymph), (b) E2 waveform of the same individual after 24 h; compare with Fig. 2(b).

start stylet penetration. At 5×10^{-4} M (35 ng per aphid) the aphids still walked, but did not accumulate near the veins. A concentration of 10^{-3} M or higher resulted in motor disfunction, but not in total paralysis, within 15 min. Death occurred within 24 h. Winged and wingless individuals responded similarly. At 10^{-4} M (7 ng per individual of 350 μg) half of the individuals recovered and resumed penetration and phloem feeding. During the first hour of feeding (Fig. 3(a)) the E2 waveforms showed a reduction mainly in the frequency of the waves (control aphids $4{\cdot}58(\pm 0{\cdot}28)\ s^{-1}$, treated aphids $2{\cdot}40(\pm 0{\cdot}20)\ s^{-1}$ with $P < 0{\cdot}01$), but after a few hours both amplitude and frequency of waves and peaks were back to normal (Fig. 3(b)), indicating that the aphids did not suffer pathological damage.

3.4 Diet feeding

When pymetrozine is administered *via* a synthetic diet in the flow chamber, feeding activities of aphids can be recorded continuously and the time lapse between first contact with the drug and final response can be accurately measured. Individuals of *M. persicae* ingest about 6 pl s^{-1} when feeding on the type of diet used in this study.[14,15] When the normal diet was replaced by one containing 300 μg pymetrozine ml^{-1}, 50% of the individuals stopped feeding after $7(\pm 0{\cdot}9)$ min. By this time, at most 2·0 ng pymetrozine per individual or 5 ng mg^{-1} aphid had been imbibed. This is less than the amount needed to disturb feeding (4–7 ng per individual or 10–18 ng mg^{-1}) when injected into the hae-

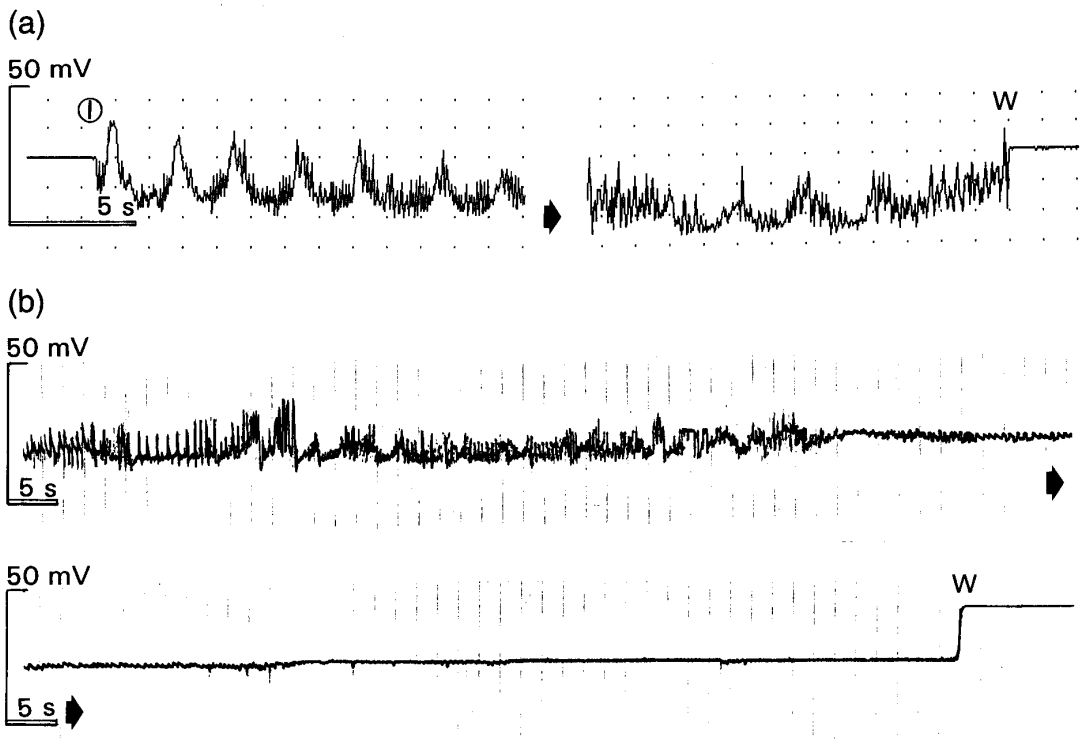

Fig. 4. (a) Computer registration of a feeding bout of *M. persicae* on an artificial diet showing stylet insertion (I), salivation and ingestion waves followed by withdrawal (W), (b) the last minutes of feeding activity on a diet containing 300 μg of pymetrozine, recorded with a chart recorder showing extinction of the waves and peaks before stylet withdrawal (W). Compare with chart recording in Fig. 2(d).

TABLE 3
Effect of Foliar Application of Pymetrozine on Penetration Behaviour of Wingless Morphs of Four Aphid Species Recorded with the EPG Technique and on Mortality and Fecundity

Aphid species (*host plant*) *Dosage* (*mg litre*$^{-}$)	*Mean value* ($\pm SE$)[a]							
	A. fabae (*broad bean*)		A. gossypii (*cucumber*)		M. euphorbiae (*potato*)		M. persicae-M_3 (*Chinese cabbage*)	
	0	*200*	*0*	*200*	*0*	*200*	*0*	*200*
Time to first penetration (min; $n = 20$)	4·5 (±2·8)	4·2 (±2·3)	1·8 (±1·6)	2·0 (±1·6)	2·5 (±1·3)	2·8 (±2·0)	1·3 (±1·4)	1·6 (±1·2)
Time to reach the phloem at first successful penetration (min)	23 (±5·7)	24 (±5·9)	20 (±9·6)	31 (±8·8)	22 (±7·5)	40 (±10·8)*	13 (±6·9)	40 (±11·3)*
Longest E2 waveform (min)	>480	42*	>480	50*	>480	58*	>480	47*
Mortality after 24 h (%; $n = 100$)	2 (±1·3)	82 (±4·7)*	3 (±1·5)	78 (±6·6)	1·5 (±1·3)	32 (±3·3)*	3 (±1·5)	70 (±4·7)*
Average daily offspring/aphid ($n = 100$)	1·9 (±0·15)	0*	2·2 (±0·16)	0*	2·3 (±0·19)	0*	2·2 (±0·15)	0*

[a] *Values statistically different from the corresponding controls (0 mg litre^{-1}) (*t*-tests, $P < 0{\cdot}05$).

molymph. Losses due to excretion are probably not relevant during this short period. The aphids did not recover within 20 h.

When wired aphids were given access to a diet already containing 300 μg pymetrozine ml^{-1} diet, all of them started probing and feeding; this was continued for the same period of time as when the drug was delivered *via* a flow system. There was still disruption of feeding when only 100 μg pymetrozine ml^{-1} was present in the diet, but in this case an average intake period of 10($\pm$1·8) min was needed. This is just significantly different from 7 min ($P < 0{\cdot}10$) and illustrates the rapid effect of pymetrozine after ingestion.

During diet feeding the pharynx pump mechanism contributes to high-amplitude electromotive force (emf) waveforms, predominantly containing a 3–4 Hz frequency (Fig. 4(a), right half). At a dose of 100 μg ml^{-1} the last minutes of the EPG registration (Fig. 4(b)) show a gradual reduction of the amplitude magnitude of these waveforms and disappearing peaks (saliva pump). Below 100 μg ml^{-1}, down to 50 μg ml^{-1} diet, feeding was sustained, although the EPGs showed a somewhat reduced amplitude of the emf waveforms.

3.5 Foliar application

On plants sprayed with 200 mg litre^{-1} pymetrozine, aphids of each species started penetration as on solvent-sprayed control plants (Table 3). There were only small differences in time between the onset of penetration and the first successful punctures of the phloem elements which resulted in a sustained E2 waveform. However, the duration of phloem sap intake (E2) was dramatically reduced at 200 mg litre^{-1} in both morphs (Table 3, wingless morph), indicating that sustained phloem sap ingestion is inhibited. For several hours the aphids can switch back to a C pattern (no phloem sap ingestion) or withdraw their stylets to resume penetration and re-enter the phloem. The final E pattern is characterized by mixtures of waves belonging to both E1 and E2 waveforms. Those of the E2 waveforms (pharynx pump) are usually small in EPGs of aphids feeding on plants, as phloem sap is under pressure and does not need to be actively imbibed.

At 80 mg litre^{-1} some honeydew was produced. Larviposition was greatly reduced, though not quite inhibited. These larvae behaved in a similar way to their parents: they seemed to accept the plants for a few hours, and subsequently stopped feeding, and died. At 200 mg litre^{-1} pymetrozine no aphids produced larvae. Death usually occurred between 24 and 48 h after settling on the treated plants, although mortality of *M. euphorbiae* was significantly lower than that of the other species ($P < 0{\cdot}01$). During periods of non-penetration the aphids often walked on the leaves with their proboscis in the resting position under the thorax.

To summarize, on plants treated with 200 mg litre^{-1} pymetrozine there was a systemic effect on penetration behaviour. Stylet penetrations did occur and small amounts of sap were consumed, but the effect on feeding behaviour was observed within a few hours; the aphids did not compensate in any way and died without producing any larvae.

3.6 Root application

On plants placed in nutrient solutions containing 40 mg litre^{-1} pymetrozine the aphids started to penetrate and the EPGs showed the normal pattern of A, B, C waveforms. In contrast to foliar application, all species showed prolonged C patterns, although the phloem phase was still shown (Table 4). The strongest effect was with *M. persicae* biotype M_2 on Chinese cabbage, where there was no phloem penetration at 100 mg litre^{-1}. It took *M. persicae* 20 h or more to show a phloem phase at 40 mg litre^{-1} and E2 waveforms seldom lasted more than 1 h (Table 4). On these plants there was a considerable interval between the first and second penetration. As on sprayed plants, the E2 waveforms were normal during the first sap intake period, but gradually they became alternate with waveforms as seen in C patterns. The aphids subsequently stopped feeding for several hours, after which they recovered and resumed penetration as observed on sprayed plants. The downward peaks of the E2 waveforms showed a low frequency, suggesting reduced salivation activity.

On plants placed in solutions with 100 mg litre^{-1} pymetrozine, the aphids again started penetration, but often the E2 waveform was not produced (Table 4). *M. euphorbiae* and *A. gossypii* still showed E2 (waveform) periods longer than 2 h, albeit of a reduced peak frequency as described before, and subsequently stopped feeding for a few hours. *A. fabae* and *M. persicae* showed only E2 periods of short duration.

The main effect of treatment of host plants with pymetrozine was the strongly reduced period of phloem feeding. *A. fabae* and *M. persicae* reacted most rapidly especially on plants where pymetrozine was taken up by the roots. Winged and wingless aphids showed the same feeding behaviour and there were only small differences in the duration of the phloem phase on treated plants (Tables 3 and 4, wingless morph). The effect of pymetrozine seemed to be morph-independent. Although stylet penetration occurred and small amounts of sap could repeatedly be consumed, food intake was insufficient for the aphids to stay alive and reproduce normally.

When aphids which had ceased feeding on plants systemically treated with 100 mg litre^{-1} pymetrozine either by foliar application or by root uptake were transferred to untreated plants they did not resume penetration. Thus, feeding behaviour was irreversibly disturbed at this concentration of pymetrozine.

TABLE 4

Effect of Root Application of Pymetrozine on Penetration Behaviour of Wingless Morphs of Four Different Aphid Species Recorded with the EPG Technique and on Mortality and Fecundity

	Mean value (±SD)[a]											
Aphid species (host plant)	A. fabae (*broad bean*)			A. gossypii (*cucumber*)			M. euphorbiae (*potato*)			M. persicae M_2 (*Chinese cabbage*)		
Dosage (mg litre^{-1})	*0*	*40*	*100*	*0*	*40*	*100*	*0*	*40*	*100*	*0*	*40*	*100*
Time to first penetration (min; $n = 20$)	4·5 (±2·5)	4·8 (±4·8)	5·2 (±3·8)	1·8 (±1·2)	1·8 (±1·0)	2·4 (±1·7)	2·5 (±1·8)	2·5 (±2·0)	4·0 (±2·2)	3·8 (±2·5)	4·2 (±3·1)	6·7 (±3·8)
Time to reach the phloem at first successful penetration (min)	20 (±6·1)	82 (±18·3)*	217 (±29·0)*	18 (±7·9)	102 (±16·7)*	149 (±25·2)*	23 (±6·3)	87 (±14·3)*	141 (±22·8)*	15 (±6·4)	>1200*	~
Interval to second penetration (min)	5 (±4·2)	18 (±10·7)	32 (±11·2)*	3 (±2·6)	14 (±5·6)*	21 (±8·1)*	3 (±1·9)	6 (±5·2)	6 (±4·8)	4 (±2·2)	>120*	>120*
Longest E2 waveform (min)	>480	205*	88*	>480	143*	165*	>480	172*	139*	>480	38*	0
Mortality after 24 h (%; $n = 100$)	2 (±1·3)	23 (±2·6)*	50 (±4·5)*	3 (±1·5)	13 (±3·7)*	57 (±5·0)*	15 (±3·4)	18 (±4·4)*	38 (±3·2)*	3 (±1·5)	67 (±4·0)*	78 (±5·7)*
Average daily offspring/aphid ($n = 100$)	1·9 (±0·15)	0·7 (±0·06)	0·2 (±0·04)	2·2 (±0·19)*	0·4 (±0·07)*	0·1 (±0·03)*	2·3 (±0·14)	0·8 (±0·06)*	0·2 (±0·04)*	1·9 (±0·15)	0·2 (±0·05)*	0

[a] *Values statistically different from the corresponding controls (0 mg litre^{-1}) (*t*-tests, $P < 0·05$).

4 DISCUSSION

The results obtained with the different application techniques provide the following information on the four possible modes of action of pymetrozine postulated in the Introduction:

Feeding site and host acceptance. At least under the present experimental conditions, pymetrozine did not stimulate the plants to produce a repellent odour nor was it repellent *per se* to any significant extent. When pymetrozine was taken up by the plants, either by the roots or after foliar application, there was no disruption of the sequence in the first steps toward host acceptance. Aphids mounted on a gold wire usually take a few minutes before they start probing. This time to first stylet penetration was not increased on treated plants.

There was a clear difference between topical application to the insects and plant treatment. Direct contact with pymetrozine resulted in an almost immediate block of the initiation of new probes. Lower dosages resulted in reduced probe initiations but the aphids approached the veins where they normally feed, suggesting that there was no disruption to feeding site location.

Stylet penetration toward the phloem. After root uptake from 100 mg litre^{-1} pymetrozine, both *A. fabae* and *M. persicae* took longer to reach the phloem whilst the other aphid species did not. Especially on Chinese cabbage treated with 100 mg litre pymetrozine, *M. persicae* repeatedly resumed penetration with an interval of a few hours, not resulting in phloem sap intake (E2 waveforms). The EPGs showed that, in all cases, a number of potential drop (pds) were produced, indicating that the aphids penetrated through the plasmalemma of parenchymous cells including those close to the phloem elements.[10] During the pds the stylets have just pierced the plasmalemma and make contact with the cell contents. There may be ingestion of small quantities of liquid[9,10,16] which, at higher pymetrozine dosages, may be sufficient to prevent access to the phloem.

Inhibition of cibarial muscle system. This would seem to be the most important effect of pymetrozine. About 10^{-4} M in the haemolymph was sufficient to reduce probing almost completely and to prevent larviposition. However, the aphids were still able to walk and, for example, to search for a suitable feeding site. It should be noted that *M. persicae* stopped ingestion a few minutes after intake of a calculated amount not exceeding 5 ng mg^{-1} aphid. Most probably, only part of this quantity circulated in the haemolymph after that period. The local concentration of pymetrozine in the oesophagus and stomach may be an order of magnitude higher before the compound is evenly distributed through the haemolymph after it has been transported across the epithelial wall of the intestine. The oesophagus passes the tritocerebrum and runs over the suboesophageal ganglion (SOG), the stomach is close to the thoracic ganglion. Probably the SOG is involved in stimulation of salivation and pharynx activities whereas the cibarial dilator muscle is innervated by the recurrent nerve of the frontal ganglion.[17] The E2 waves are of emf origin and reflect activity of the cibarial valves and pump muscles.[11,12] The main effects observed during the E2 waveforms were: (1) an increased irregularity and a decreased amplitude of the E2 (small) waves and (2) decreased frequency and amplitude of the E2 peaks (Figs 3(a), 4(b)). Thus, the activity of the cibarial valves, the food pump and the salivary pump seems to be affected by pymetrozine. Since the nature of these changes differs and appears not only to affect muscle contraction as such, but also the number of contractions per unit of time, the mode of action seems more central than the efferent nerves and muscles. Phloem sap is under pressure and activity of the pharyngeal pump system is not essential once the stylets have entered a phloem vessel and some watery saliva has been injected.[8–10] Nevertheless the aphids stopped ingestion after intake of pymetrozine during the production of E2 waveforms on plants. Moreover, on plants that had been systematically treated there seemed to be an increasing reluctance to sustain E2 waveforms and subsequent probing after stylet withdrawal.

Toxic effects on the nervous system. When aphids do not feed, as may happen during host plant alternation, mortality will increase within 24 h.[16] Mortality and reproduction rate of aphids feeding on plants treated with the highest dose of pymetrozine (Table 3) were of similar magnitude to those of aphids unable to feed, but free to move on a potential host plant;[18] the majority of these latter died within 48 h. Therefore, the effect would not seem to be that of a toxic compound that inhibits basic metabolic processes or the general nervous system. During most of that period they remained able to walk and even to select veins protruding from the leaf surface.

Summarizing, the results presented provide the view that pymetrozine does not exhibit general toxic action in aphids at an effective dosage, but interferes with normal feeding activities by affecting the nervous regulation of fluid intake. There are no repellent or antifeedant effects that could explain the inhibition of feeding. Stylet insertion and feeding still occur on diet containing 300 μg ml^{-1} pymetrozine until a haemolymph level is reached equivalent to effective concentration after microinjection. This property of pymetrozine is in contrast to the concentration-dependent change of the mode of action of the insecticide imidacloprid on *M. persicae*.[19] At low doses, this insecticide evokes a reversible antifeedant response, but at higher doses irreversible symptoms of toxic effects were observed.[20] Although 50–100 μg ml^{-1} pymetrozine in artificial diets

seem to affect the feeding mechanism, feeding is sustained without interruption. Lower doses simply do not have any effect.

Under field conditions, the aphids can be confronted with pymetrozine applied both topically, in curative treatments, and systematically, in preventive use of the aphicide. This depends on the crop that is to be protected in relation to flight behaviour of the important aphid species and on seasonal effects.[1,6,13,21]

The aphid species *M. persicae* and *M. euphorbiae* are vectors of both persistent and non-persistent viruses.[13,22] A number of control measures can be taken to protect the crop against aphids.[23] However, the effectiveness of insecticides in reducing virus transmission is limited, since aphids will probe and feed for some time on treated plants unless the process of host acceptance is modified in an early phase. Pymetrozine strongly reduces virus transmission of potato leafroll luteovirus (PLRV) but also considerably reduces transmission of potato Y potyvirus, necrotic strain (PVY^N) in laboratory experiments.[24] Feeding on phloem sap is essential to acquire and transmit PLRV. On pymetrozine-treated plants there is no sustained feeding. The significant reduction in PVY^N transmission may be caused by the limited number of cell penetrations on treated plants.[24]

In conclusion, the mode of action of pymetrozine in aphids seems to be new; no other aphicide is known to cause a selective inhibition of feeding without paralyzing the insect. This effect is most probably exerted *via* the nervous system. More details of the cellular action of pymetrozine are currently being studied.

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